iGEM 2016: Pittsburgh
Week 8 Lab Notebook

Monday, July 11

Lab meeting

Anneal oligos, edited versions only (last week's got loading dye) (Claire)

25 nM : 50 nM	0.5 uL 2.5 uM substrate
	1 uL 2.5 uM DNAzyme
	5 uL 10X T4 DNA Ligase Buffer
	43.5 uL water
50 nM : 50 nM	1 uL 2.5 uM substrate
	1 uL 2.5 uM DNAzyme
	5 uL 10X T4 DNA Ligase Buffer
	43 uL water
50 nM : 75 nM	1 uL 2.5 uM substrate
	1.5 uL 2.5 uM DNAzyme
	5 uL 10X T4 DNA Ligase Buffer
	42.5 uL water
50 nM : 100 nM	1 uL 2.5 uM substrate
	2 uL 2.5 uM DNAzyme
	5 uL 10X T4 DNA Ligase Buffer
	42 uL water

Repeat cell-free Collins plasmids with annealed oligos (Claire and Praneeth)

Dilute all DNA to 50 nM

D switch

	Con-trol	Mis-Match	DNA trigger	Dup. 0.5:1	Duplex 1:1	Duplex 1:1.5	Duplex 1:2	PO strand	Enzymatic strand
Sol'n A	2	2	2	2	2	2	2	2	2
Sol'n B	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
RNAse inhibitor	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sub-strate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
switch	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
trigger	0	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
water	0.4	0	0	0	0	0	0	0	0

G switch

	Con-trol	Mis-Match	DNA trigger	Dup. 0.5:1	Duplex 1:1	Duplex 1:1.5	Duplex 1:2	PO strand	Enzymatic strand
Sol'n A	2	2	2	2	2	2	2	2	2
Sol'n B	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
RNAse inhibitor	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sub-strate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
switch	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
trigger	0	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
water	0.48	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08

34 reactions

Master (36 rxns): 76 uL Solution A
57 uL Solution B
9.5 uL RNAse inhibitor
9.5 uL substrate

Separate into 2 x 76 uL

D	//	G
Add 11.4 uL	//	9.88 uL switch DNA
Remove 9.2 uL	//	9.04 uL for controls
Add 0.8 uL	//	0.96 uL water

2 CONTROLS DONE

Add 1.36 uL water

Separate into 7 x 9.2 uL

Add 0.8 uL appropriate DNA

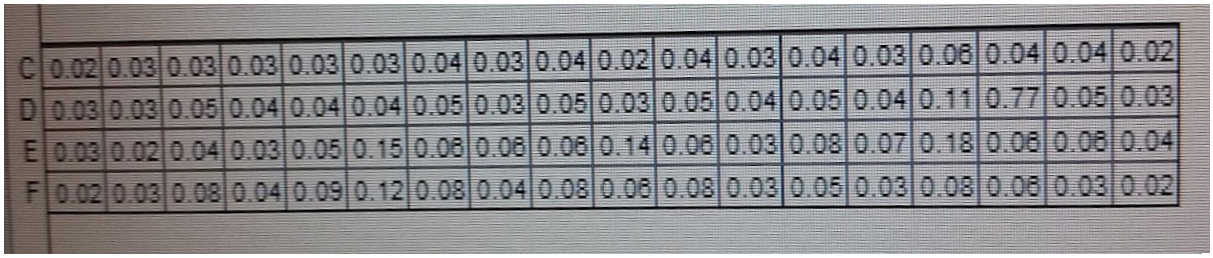
Pipette 5 uL of each reaction into plate well, then incubate 2 hr

Claire and Praneeth each do duplicates

Switch D ran out for Claire. For 11.4 uL, 6 uL original
1.4 uL 13-2 (83.6 ng/uL)
4 uL last year's (19.9 ng/uL)

C and D Praneeth; E and F Claire. C and E D switch; D and F G switch. Duplicates following charts above.

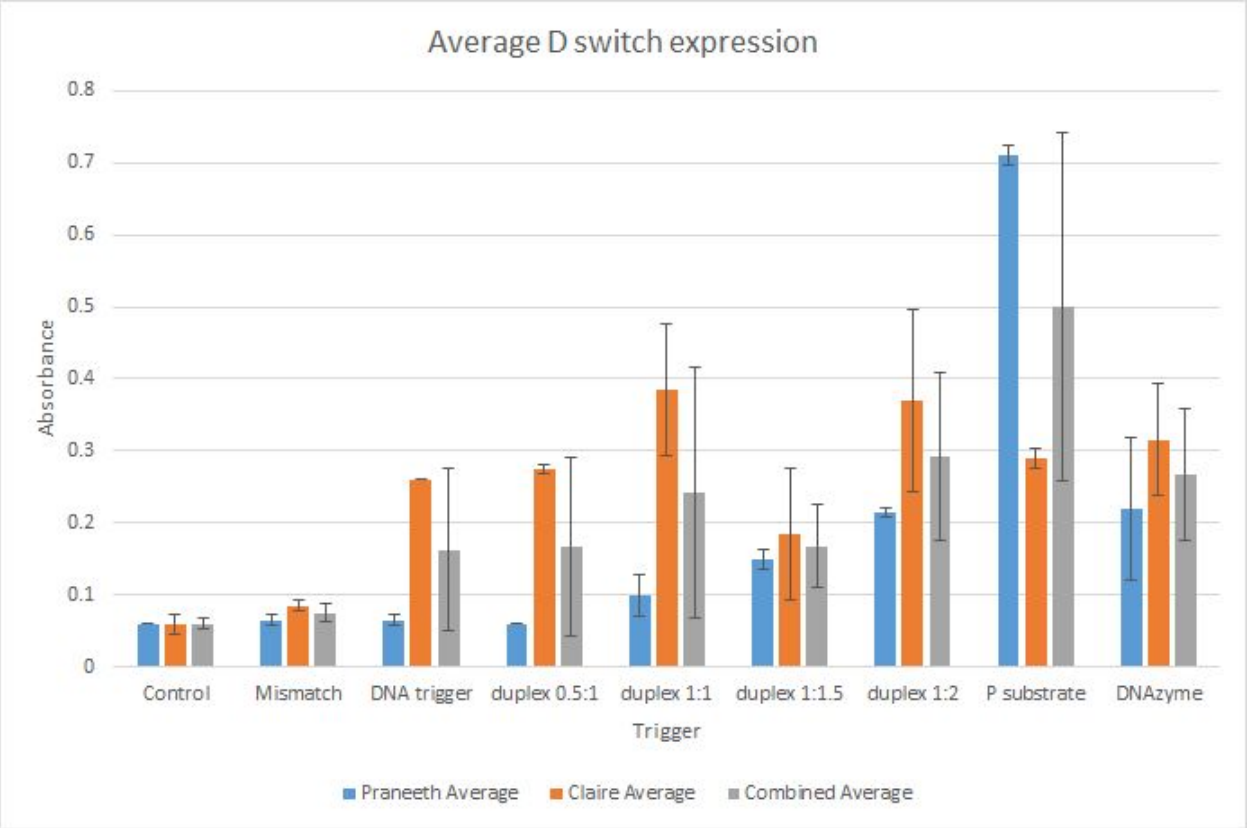
No dilution:



C	0.02	0.03	0.03	0.03	0.03	0.03	0.04	0.03	0.04	0.02	0.04	0.03	0.04	0.03	0.06	0.04	0.04	0.02
D	0.03	0.03	0.05	0.04	0.04	0.04	0.05	0.03	0.05	0.03	0.05	0.04	0.05	0.04	0.11	0.77	0.05	0.03
E	0.03	0.02	0.04	0.03	0.05	0.15	0.06	0.06	0.06	0.14	0.06	0.03	0.08	0.07	0.18	0.06	0.06	0.04
F	0.02	0.03	0.08	0.04	0.09	0.12	0.08	0.04	0.08	0.06	0.08	0.03	0.05	0.03	0.08	0.08	0.03	0.02

Dilution to 10 uL, D switch:

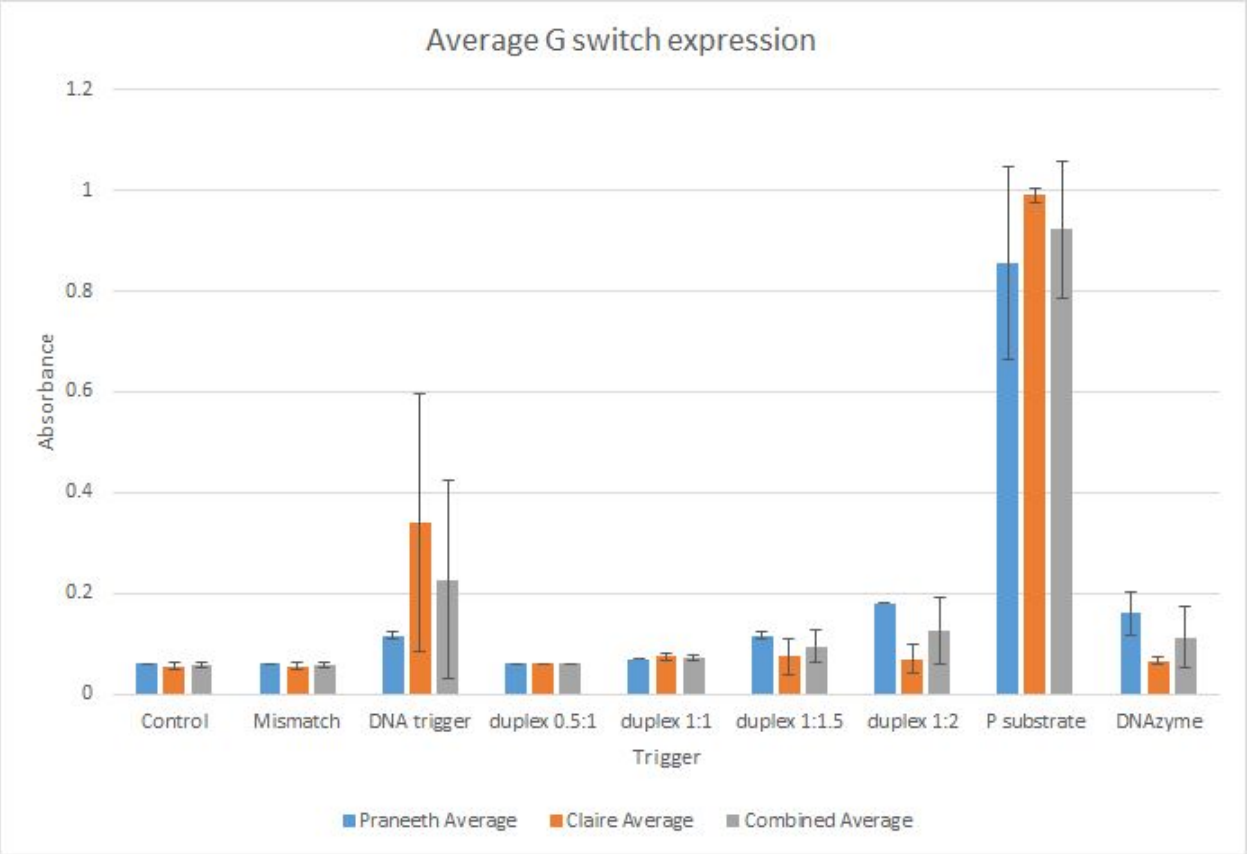
	Praneeth		Claire		Praneeth		Claire		Combined	
	Absorb 1	Absorb 2	Absorb 1	Absorb 2	Average	St Dev	Average	St Dev	Average	St Dev
Control	0.06	0.06	0.07	0.05	0.06	0	0.06	0.014142	0.06	0.008165
Mismatch	0.07	0.06	0.09	0.08	0.065	0.007071	0.085	0.007071	0.075	0.01291
DNA trigger	0.07	0.06	0.26	0.26	0.065	0.007071	0.26	0	0.1625	0.112657
duplex 0.5:1	0.06	0.06	0.27	0.28	0.06	0	0.275	0.007071	0.1675	0.124197
duplex 1:1	0.12	0.08	0.45	0.32	0.1	0.028284	0.385	0.091924	0.2425	0.173662
duplex 1:1.5	0.16	0.14	0.25	0.12	0.15	0.014142	0.185	0.091924	0.1675	0.057373
duplex 1:2	0.22	0.21	0.46	0.28	0.215	0.007071	0.37	0.127279	0.2925	0.115866
P substrate	0.72	0.7	0.3	0.28	0.71	0.014142	0.29	0.014142	0.5	0.242762
DNAzyme	0.29	0.15	0.37	0.26	0.22	0.098995	0.315	0.077782	0.2675	0.091059



Absorbance read at 570 nM. 25 ng of switch (Claire ran low) paired with 4 nM of different triggers. Reactions incubated as 5 uL reactions in plate.

Dilution to 10 uL, G switch

	Praneeth		Claire		Praneeth		Claire		Combined	
	Absorb 1	Absorb 2	Absorb 1	Absorb 2	Average	St Dev	Average	St Dev	Average	St Dev
Control	0.06	0.06	0.06	0.05	0.06	0	0.055	0.007071	0.0575	0.005
Mismatch	0.06	0.06	0.05	0.06	0.06	0	0.055	0.007071	0.0575	0.005
DNA trigger	0.11	0.12	0.16	0.52	0.115	0.007071	0.34	0.254558	0.2275	0.196193
duplex 0.5:1	0.06	0.06	0.06	0.06	0.06	0	0.06	0	0.06	0
duplex 1:1	0.07	0.07	0.08	0.07	0.07	0	0.075	0.007071	0.0725	0.005
duplex 1:1.5	0.11	0.12	0.1	0.05	0.115	0.007071	0.075	0.035355	0.095	0.031091
duplex 1:2	0.18	0.18	0.09	0.05	0.18	0	0.07	0.028284	0.125	0.065574
P substrate	0.72	0.99	1	0.98	0.855	0.190919	0.99	0.014142	0.9225	0.135247
DNAzyme	0.19	0.13	0.07	0.06	0.16	0.042426	0.065	0.007071	0.1125	0.060208



Absorbance read at 570 nM. 25 ng of switch paired with 4 nM of different triggers. Reactions incubated as 5 uL reactions in plate.

Transform D and G switch plasmids (Maddie)

Liquid cultures of amilCP-terminator ligation plates (Claire and Praneeth)

Tuesday, July 12

PCR eGFP with VF and VR primers (Claire)

50 uL reaction

32.5 uL water
10 uL 5x Phusion buffer
1 uL dNTP's
2.5 uL VF2
2.5 uL VR
1 uL template
0.5 uL DNA polymerase

Miniprep amilCP liquid cultures (Praneeth)

Concentrations (ng/uL)

1	61.4	8	141.0	14	61.8
2	61.5	9	120.4		
4	85.9	10	124.2		
5	110.8	11	81.3		
6	106.8	12	104.8		
7	153.0	13	61.8		

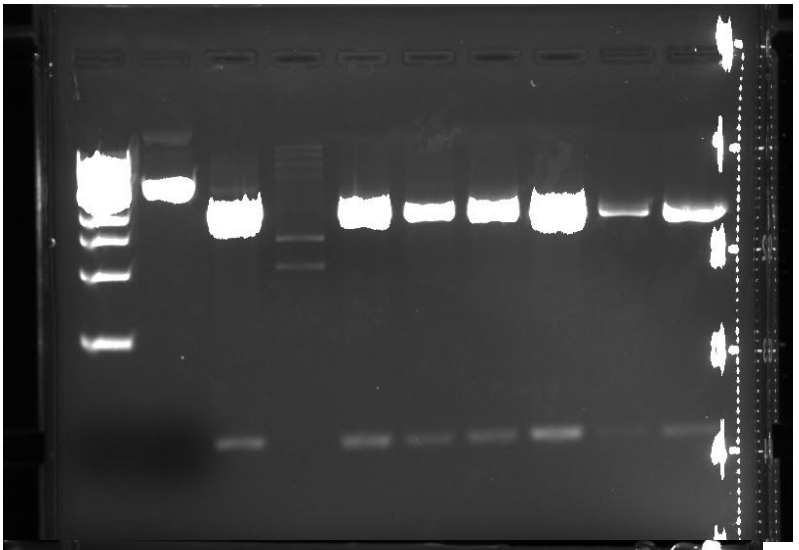
Digest (Praneeth)

0.5 uL EcoRI
0.5 uL PstI
1.0 uL buffer

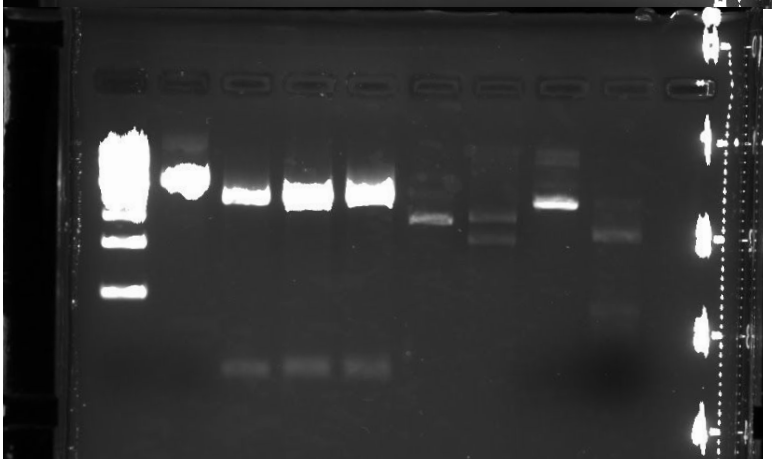
1 ug DNA =						
	1	16.29	8	7.09	14	16.29
	2	16.29	9	8.31		
	4	11.64	10	8.05		
	5	9.02	11	12.3		
	6	9.36	12	9.54		
	7	6.54	13	7.91		

H2O to 10 uL = 7: 1.46 8: 0.91 13: 0.09

Gel (Praneeth)



Lane	1	ladder
	2	uncut amilCP
	3	1
	4	2
	5	4
	6	5
	7	6
	8	7
	9	8
	10	9



Lane	1	ladder
	2	uncut amilCP
	3	10
	4	11
	5	12
	6	13
	7	14
	8	uncut eGFP
	9	PCR product

amilCP 2 and 14 promising
PCR unsuccessful

Purify PCR reaction (Maya)

Concentration = 63.6 ng/uL

Transform amilCP 2 and 14 into T7 cells (Claire)

Transform switch plasmids again; plates didn't grow overnight (Maddie)

Make DNAzyme B buffer (Claire and Maya)

0.365 g NaCl (25 mM)
2.616 g MOPS (50 mM)
NaOH to pH 7.5

H2O to 250 mL

Make EDTA (Claire and Maya)

1.461 g EDTA
NaOH to pH 8.0
H2O to 10 mL

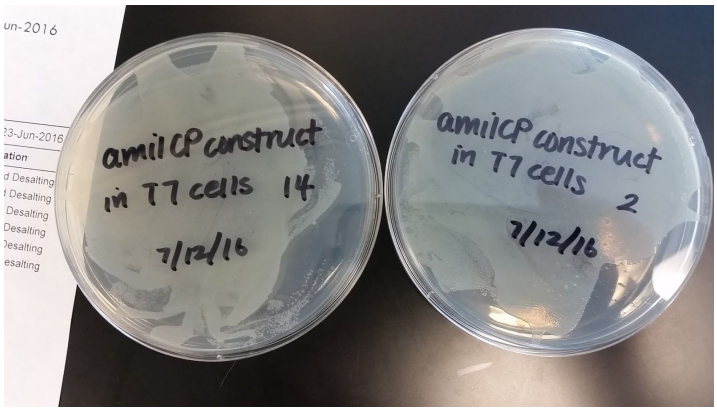
Make 10X TBE buffer (Claire and Maya)

10.8 g Tris base
5.5 g boric acid
4 mL EDTA at pH 8.0
Water to 100 mL

Make 1X PBS (Claire and Maya)

0.4 g NaCl
0.01 g KCl
0.072 g Na_2HPO_4
0.012 KH_2PO_4
HCl to pH 7.4
H2O to 50 mL

Wednesday, June 13



amilCP plates not blue

Digest amilCP with XbaI and PstI (Maya)

1 uL XbaI
1 uL SpeI (not sure which used)
2 uL buffer
2 ug DNA = 8.16 g PP amilCP
7.84 uL H2O

Digest T7-RBS with SpeI and PstI (Maya)

1 uL PstI
1 uL SpeI
2 uL buffer
2 ug DNA = 9.22 g (concentration 108.5 ng/uL)
6.78 uL H2O

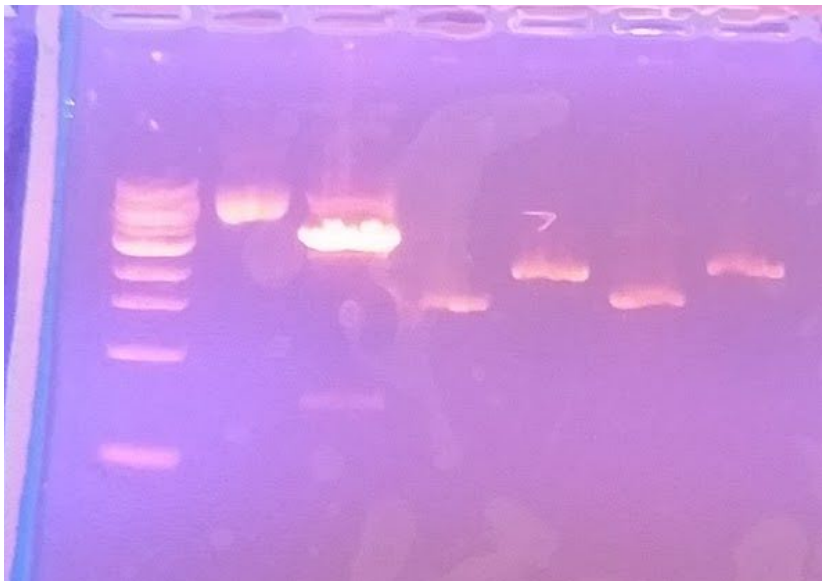
Digest terminator with EcoRI and XbaI (Maya)

1 uL XbaI
1 uL EcoRI
2 uL buffer
2 ug DNA = 10.32 uL DNA (concentration 96.9 ng/uL)
5.68 uL H2O

Gel (Maya)

Lane		
	1	ladder
	2	uncut amilCP
	3	digested amilCP
	4	uncut T7
	5	T7 cut

6 uncut terminator
7 terminator cut



Purify terminator and promoter (Maya)
Concentrations: promoter 13.9 ng/uL
Extract amilCP band (Maya)
Ligate to promoter (Maya)

1:1	plasmid	1 uL
	Insert	7.32 uL
	Buffer	2 uL
	Ligase	1 uL
	Water	8.68 uL

Digest amilCP again for higher concentration (Maya)

40 uL reaction	2 uL XbaI
	2 uL PstI
	4 uL buffer
	5 ug DNA
	11.3 uL water

Extract amilCP band
Concentration = 66.4 ng/uL
Liquid cultures of Collins plasmids (Maddie)
Plate glycerol stock of T7-amilCP-terminator (Claire)
Anneal edited G DNAzyme strands at 10 uM (Praneeth)

5 uM : 10 uM	2.5 uL 100 uM substrate
	5 uL 100 uM catalytic
	5 uL T4 ligase buffer
	37.5 uL water
10 uM : 10 uM	5 uL 100 uM substrate
	5 uL 100 uM catalytic

	5 uL T4 ligase buffer
	35 uL water
10 uM : 15 uM	5 uL 100 uM substrate
	7.5 uL 100 uM catalytic
	5 uL T4 ligase buffer
	32.5 uL water
10 uM : 20 uM	5 uL 100 uM substrate
	10 uL 100 uM catalytic
	5 uL T4 ligase buffer
	30 uL water

Erbium solution and dilution (Claire)

1 M Er^{+3} = 0.095 g in 250 uL water

1 mM = 1:1000

DNAzyme cleavage with erbium (8 reactions, 50 uL) (Praneeth)

3.5 uL 10 uM annealed duplex (x4 different ratios) = 0.7 uM

5 uL 1 mM Er^{+3} = 100 uM OR 0.5 uL 1 mM Er^{+3} = 10 uM

41.5 uL buffer B OR 46 uL buffer B

Incubate at room temperature for 20 minutes

Quench with 5 uL loading buffer

dPAGE (1X TBE buffer) (Praneeth and Claire)

Load about 40 uL of each reaction

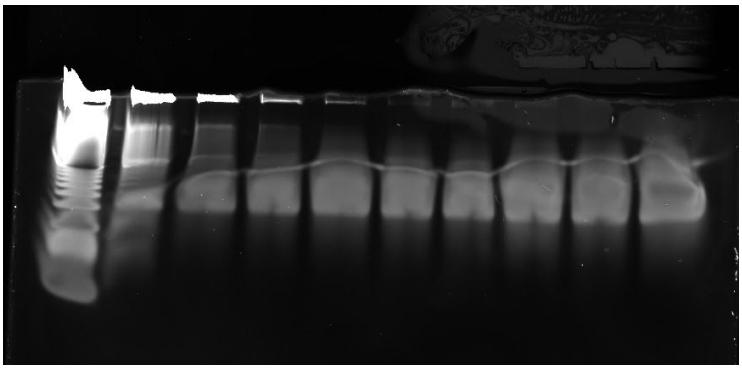
Lane	1	10 bp ladder (5 uL + 2 uL dye)	6	0.5 : 1 100 uM Er^{+3}
	2	0.5 : 1 10 uM Er^{+3}	7	1 : 1
	3	1 : 1	8	1 : 1.5
	4	1 : 1.5	9	1 : 2
	5	1 : 2	10	loading buffer

Probably unequal amounts of each reaction in each lane

Run at 100 volts, 45 min

Cover with 1X SYBR Gold (50 mL), shaker for 15 min

Bands didn't separate enough



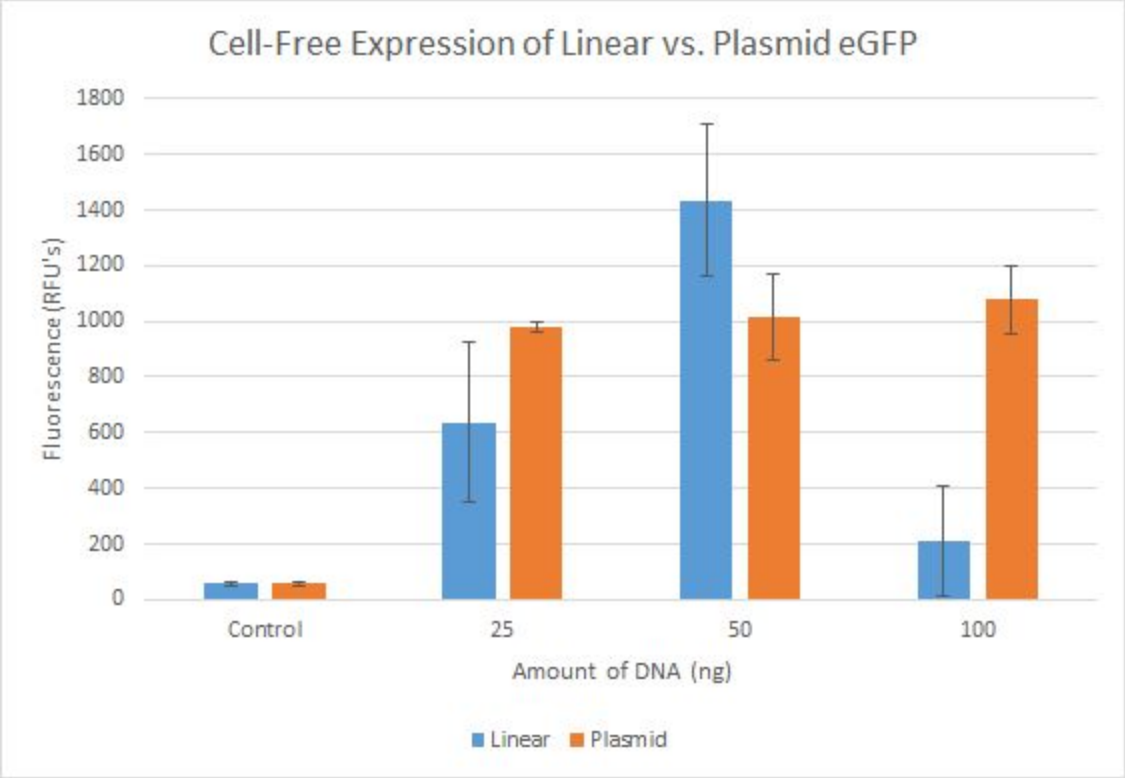
Thursday, July 14

Camp BioE presentation by Maddie, Praneeth, and Claire

Miniprep liquid cultures (Maya)

Cell-free extract reaction with GFP linear vs plasmid (Maya)

	Linear				Plasmid			
	1	2	Avg	St Dev	1	2	Avg	St Dev
Control	59.467	53.25	56.3585	4.40	59.467	53.25	56.3585	4.40
25	839.558	431.962	635.76	288.214	990.495	965.826	978.1605	17.444
50	1242.428	1626.218	1434.323	271.381	908.941	1126.075	1017.508	153.537
100	349.986	70.458	210.222	197.656	993.085	1165.086	1079.086	121.623



Ligate newer amilCP to promoter (Claire)

	control	3:1	7:1
plasmid	1 uL	5 uL (lower conc.)	1 uL
insert	0	0.75	1.73
buffer	2 uL	2 uL	2 uL

ligase	1 uL	1 uL	1 uL
water	16 uL	3.25 uL	14.27 uL

Transform in 66 uL Cheryl's cells (Praneeth)
dPAGE gel of yesterday's reactions (Claire and Praneeth)
5 uL reaction + 5 uL loading buffer

Lane	1	10 bp ladder (5 uL + 2 uL dye)	6	1 : 1 100 uM Er^{+3}
	2	0.5 : 1 10 uM Er^{+3}	7	1 : 1.5
	3	1 : 1.5	8	1 : 2
	4	1 : 2	9	10 uL loading buffer
	5	0.5 : 1 100 uM Er^{+3}	10	10 uL loading buffer

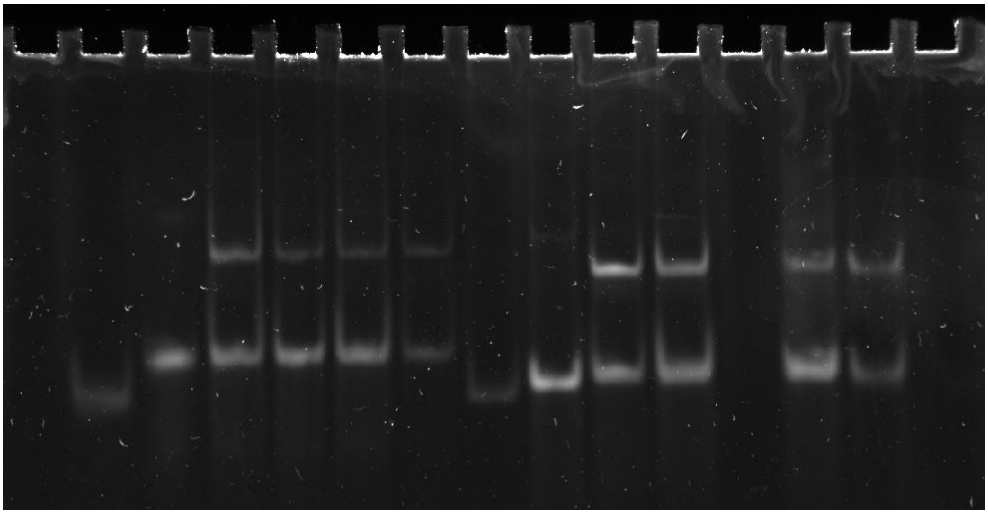
Run at 100 volts for 1 hr 15 min
Gel broke while removing from plate
Liquid cultures of T7-amilCP ligation and T7-amilCP-terminator (Maddie)
LB and CM plates (Claire)
1 L 10X TBE buffer (Praneeth and Claire)

Friday, July 15

Native polyacrylamide gel of edited DNAzyme duplexes (Claire and Nick)
15% gel
For duplexes, add 4 uL loading buffer (no dye) to 16 uL of 50 nM duplex
For controls, add 2 uL buffer to 10 uL 50 nM oligo
Load 12 uL in each well

Gel	Lane	2	G 0.5:1	8	D 0.5:1
		3	G 1:1	9	D 1:1
		4	G 1:1.5	10	D 1:1.5
		5	G 1:2	11	D 1:2
		6	G P substrate	13	D P substrate
		7	G DNAzyme	14	D DNAzyme

Run at 100 V for 1 hour
Remove comb "fingers" and cut left corner
Stain in 1X SYBR Gold for 20 min
Rinse with water 2-3 times before imaging to remove stain



DNAzyme duplexes with erbium (Praneeth)

25 uL reactions, edited G, 4 ratios

0.7 uM annealed duplexes

100 uM or 10 uM Er^{+3}

Buffer B to volume

Incubate 20 minutes at room temperature

Quench with 25 uL loading buffer

Heat at 95 degC for 5 minutes; load gel while hot

Lane	1	1 kb ladder	6	0.5:1 100 uM
	2	0.5:1 10 uM	7	1:1
	3	1:1	8	1:1.5
	4	1:1.5	9	1:2
	5	1:2	10	loading buffer

Run at 100V for 1 hour 30 minutes

Stain with SYBR Gold for 40 min

Rinse before imaging

Miniprep liquid cultures (Maddie)

Concentrations:

Digest check of ligations (Maya)

T7-amilCP: EcoRI and PstI, 5ul reactions

.25 ul of each enzyme

.5 ul of buffer

Colony

1: DNA - 0.7220 ul
H2O - 3.278 ul

2: DNA - 0.7375 ul
H2O - 3.263 ul

3: DNA - 0.6949 ul
H2O - 3.3051 ul

4: DNA - 0.7782 ul
H2O - 3.2218 ul

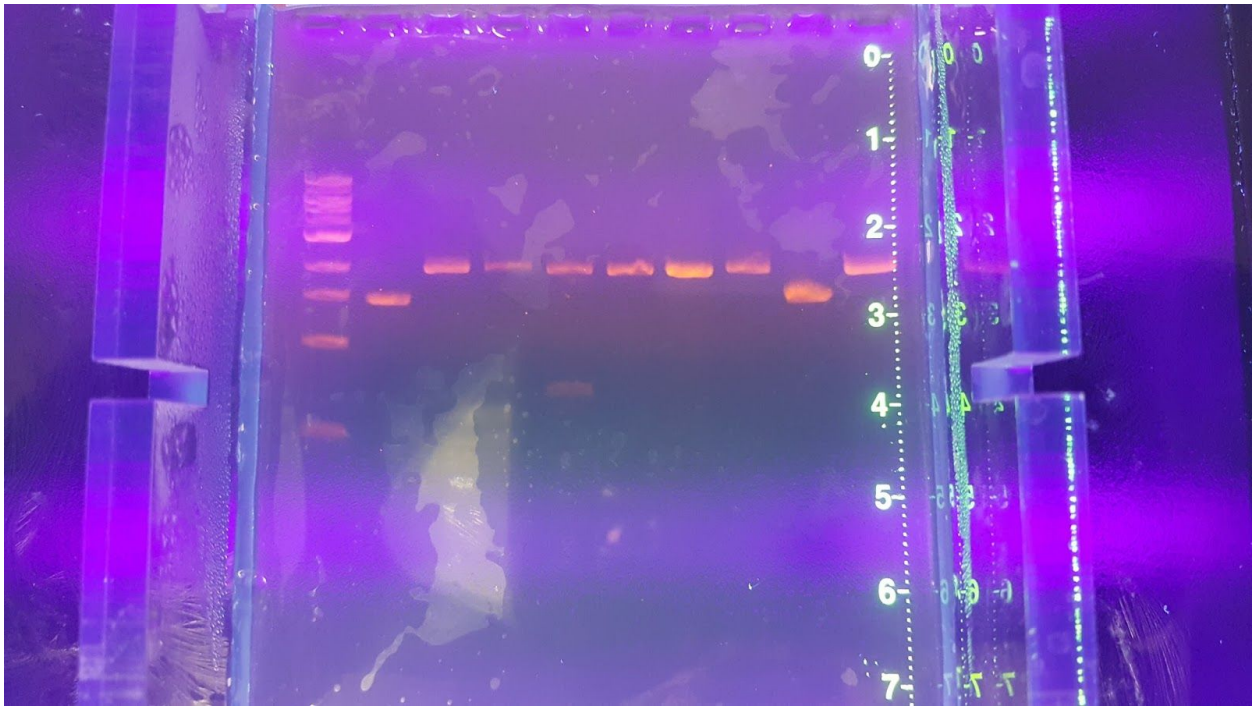
5: DNA - 1.8726 ul
H2O - 2.1274 ul

6: DNA - 0.6689 ul
H2O - 3.3311 ul

T7-amilCP-Terminator: EcoRI and PstI (Maddie)

Gel (Maya)

Lane	1	1kb ladder	6	Colony 4
	2	T7 uncut control	7	Colony 5
	3	Colony 1	8	Colony 6
	4	Colony 2	9	Term Uncut Control
	5	Colony 3	10	T7-amilCP-Term



Colony 3 (Lane 5) has a second band that indicates successful ligation, will proceed with final ligation on Monday

Rest of them do not

T7-amilCP-Term from glycerol stocks did not have second band - not successful

Sunday, July 17

Liquid cultures of promoter-new amilCP ligations (Maddie)